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A DISJUNCT POPULATION OF *EUCALYPTUS GLOBULUS* SSP. *BICOSTATA* FROM SOUTH AUSTRALIA

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Summary

VAILLANCOURT, R. E., BOOMSMA, D. B. & NICOLLE, D. (2001) A disjunct population of *Eucalyptus globulus* ssp. *bicostata* from South Australia. *Trans. R. Soc. S. Aust.* **125** (1), 65-68, 31 May, 2001.

A population of *Eucalyptus globulus* ssp. *bicostata* was recently discovered at Mt Bryan (SA) which is more than 600 km from the nearest other population of this taxon. The aim of this study was to determine whether this population is natural or whether it might have been planted after the arrival of pastoralists to the area. To achieve this aim we used RAPD molecular marker analysis of a large (10 m diam) lignotuberous stand of *E. globulus* ssp. *bicostata* that roughly formed a ring. The RAPD analysis indicated no differences between samples taken from the lignotuberous stand, although individuals from outside it were all different from it and from one another. Because the lignotuberous stand of *E. globulus* ssp. *bicostata* is likely to originate from a single individual and is very large, it is likely to be very old (possibly as old as 4000 years) and this would imply that the population was not established by pastoralists. How did the *E. globulus* ssp. *bicostata* become established on Mt Bryan? Four possibilities are discussed, namely; natural long distance seed dispersal, seed dispersal by humans before the arrival of pastoralists, long distance pollen dispersal and connection to the Victorian *Eucalyptus globulus* ssp. *bicostata* forest in the past.

KEY WORDS: Lignotuber, clone size, RAPD, fingerprinting.

Introduction

A population of *Eucalyptus globulus* ssp. *bicostata* (Maiden, Blakely & J. Simm.) Kirkpatr. was recently discovered at Mt Bryan SA (33° 26′ S, 138° 57′ E) by B. Bates. This population is unusual in that it is more than 600 km from the nearest known *E. globulus* ssp. *bicostata* population (Otway Ranges, Victoria) and is the only population of that species west of the Murray-Darling drainage system. The population is situated on the slopes of a high ridge south-west of the summit of Mt Bryan, at an altitude between 680 and 890 m. The entire population consists of approximately 80 apparently very old, large individuals and between 160 and 180 "sapling stage" individuals with a stem diameter of less than 300 mm just above ground level. Small seedlings at the cotyledon to the fifth leaf-pair stage were observed at the site in 1996/97 but seedlings were not observed in August 2000. They may have been removed by sheep. The population has a range of approximately 1000 m and forms three sub-populations separated by c. 200 m each, the western sub-population being the largest. Sapling stage individuals were more plentiful in, although not restricted to, the relatively lower elevations within the population. The *E. globulus* ssp. *bicostata* trees ranged in height from less than 5 to 18 m. The understorey was dominated by native grasses and herbs, although some *Allocasuarina verticillata* (Lam.) L. Johnson and *Bursaria spinosa* Cav. occurred within the population. Six plant species occurring at the site are classified as rare or endangered, namely, *Asplenium flabellifolium* Cav., *Derwentia decorosa* (F. Muell.) B. G. Briggs & Ehrend., *Hymenanthera dentata* R. Br. ex DC., *Lepidium pseudo-tasmanicum* Thell., *Olearia pannosa* Hook. ssp. *pannosa*, and *Rhodanthe anthemoides* (Sprengel) Paul G. Wilson (P. J. Lang, pers. comm. 2000). No other eucalypts occurred with *Eucalyptus globulus* ssp. *bicostata*. Further down Mt Bryan the ssp. *bicostata* population is grassland down to midway on the south-western slope. Below this grassland is open *E. leucoxylon* F. Muell. /*E. porosa* F. Muell. ex Miq. /*A. verticillata* woodland. The local area is one of the coldest in South Australia, with the nearest temperature-recording weather station at Yongala recording average winter minima of 2.5° C and an extreme (July) minimum of minus 8.2° C, the lowest in SA (Bureau of Meteorology; http://www.BoM.GOV.AU/climate/).

In eucalypts, vegetative propagation occurs through lignotubers. A lignotuber is a semi-subterranean woody mass of stem-like tissue that gives protection to a large reserve of epicormic buds. These allow rapid regeneration after stem destruction or damage by fire or other causes (Jacobs 1955;

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Chattaway 1958). Lignotubers occur in the majority of *Eucalyptus* (L'Hérit.) species at some stage in their life cycle (Jacobs 1955). Repeated damage to a tree can result in extensive lignotuber development and formation of a multi-stemmed stand (Lacey & Johnston 1990). Eucalypts capable of vegetative regrowth can live longer than single-stemmed trees (Tyson *et al.* 1998).

The question has been raised as to whether this South Australian population of *E. globulus* ssp. *bicostata* is natural or whether it might have been planted after the arrival of pastoralists to the area. A large stand of *E. globulus* ssp. *bicostata* that roughly formed a ring shape was found at the site at about 850 m altitude in the western sub-population. This stand is very large, being 10 m in diameter and potentially could have arisen from lignotuberous growth. Other lignotuberous stands of a similar size and possibly even larger are also present at the site, but are more difficult to identify because of lignotuber fragmentation and non-circular development of the stand.

Molecular markers are essential in identifying individual genotypes and studying vegetative propagation because the clonal nature of some vegetation cannot be established with confidence by morphological assessment alone. Random Amplified Polymorphic DNA (RAPD) (Williams *et al.* 1990; Welsh & McClelland 1990) is a useful type of molecular marker for the study of genetic variation since numerous loci can be sampled. RAPD analysis has been used extensively in eucalypts, in detecting differences between closely related species and hybrids (Sale *et al.* 1996; Rossetto *et al.* 1997), in studies of genetic diversity and population structure (Nesbitt *et al.* 1995; Skabo *et al.* 1998), in fingerprinting studies (Keil & Griffin 1994; Nesbitt *et al.* 1997; Vaillancourt & Skabo 1999), in studies of breeding systems (Gaiotto *et al.* 1997) and in studies of vegetative propagation by lignotuber (Kennington *et al.* 1996; Tyson *et al.* 1998; Rossetto *et al.* 1999). The aim of this study was to determine whether the large lignotuberous *E. globulus* ssp. *bicostata* stand is clonal. If it is, then its large size would imply that it is very old suggesting that the population could not have been established by pastoralists.

Materials and Methods

Mature adult leaf material from eight *Eucalyptus globulus* ssp. *bicostata* samples was weighed and frozen in liquid nitrogen prior to use. Four of these samples were from the possible clone and four other samples came from trees away from the lignotuberous stand. The four samples from the possible clone came from the four cardinal points of the lignotuber. Total genomic DNA was isolated from 2.0 g of leaf material according to the CTAB method of Doyle & Doyle (1990).

The DNA from each tree was assayed for Random Amplified Polymorphic DNA (RAPD) markers (Welsh & McClelland 1990; Williams *et al.* 1990). Amplification conditions were as in Nesbitt *et al.* (1997). Primers were obtained from Operon Technologies Inc. (10000 Atlantic Ave., Alameda CA 94501 USA) and the University of British Columbia (6174 University Boulevard, Vancouver, B.C. V6T 1Z3). Twenty-four primers previously shown to produce polymorphic bands (Vaillancourt & Skabo 1999) were used: OPA-02, OPA-14, OPA-15, OPA-17, OPA-20, OPB-05, OPC-19, OPD-05, OPE-07, OPF-04, UBC 30, UBC 210, UBC 215, UBC 217, UBC 218, UBC 232, UBC 234, UBC 237, UBC 243, UBC 249, UBC 266 and UBC 290. Amplified fragments were electrophoretically separated in a 1.5% w/v agarose gel, using 1 X TBE buffer, and photographed after staining with ethidium bromide. Consistency of interpretation was established by repeating three samples with each primer. In general bands were not scored if they were faint or diffuse, or occurred in the extremes of the amplified size range. Only bands that were present in 25% to 75% of the samples were used in the analysis, as reported in Skabo *et al.* 1998.

The presence/absence of RAPD bands was used to calculate a similarity matrix of simple matching coefficients (Sokal & Sneath 1963), using the NTSYS program (Rohlf 1993). The simple matching coefficient (SM) is defined as the total number of matches (shared absence or presence) between two individuals, divided by the total number of bands scored. The same program was then used to calculate the clustering of trees with the UPGMA algorithm and a dendrogram showing the relatedness of the samples was produced.

Results and Discussion

Forty eight polymorphic bands that met our selection criteria were scored for the eight DNA samples. Samples 1-4 from the possible lignotuberous stand were identical with a similarity of 1.0 (Table 1). Samples 5-8 were all different from one another and from samples of the lignotuberous stand (Fig. 1). The tree most closely related to the lignotuberous stand, tree 5, joined the lignotuberous stand samples at a level (SM = 0.58) that shows that it is not closely related to it. Nesbitt *et al.* (1997) found that RAPD variation within clones was trivial compared to the variation found even between full-siblings and that similarity decreased with pedigree distance. The lack of any variation between samples from the lignotuberous stand and the much lower degree of similarity with the rest of the samples, over

TABLE 1. *Simple matching coefficient (SM) measure of similarity between samples from a* Eucalyptus globulus *ssp.* bicostata *population at Mt Bryan in South Australia calculated with RAPD markers.*

no.	Sample number (no.) 1	2	3	4	5	6	7	8
1	1.00							
2	1.00	1.00						
3	1.00	1.00	1.00					
4	1.00	1.00	1.00	1.00				
5	0.61	0.59	0.57	0.57	1.00			
6	0.34	0.35	0.34	0.34	0.47	1.00		
7	0.43	0.43	0.43	0.43	0.40	0.40	1.00	
8	0.32	0.33	0.34	0.34	0.38	0.49	0.66	1.00

Samples 1-4 are from the 10m wide lignotuberous stand, while samples 5-8 are from individual trees in the vicinity of the stand.

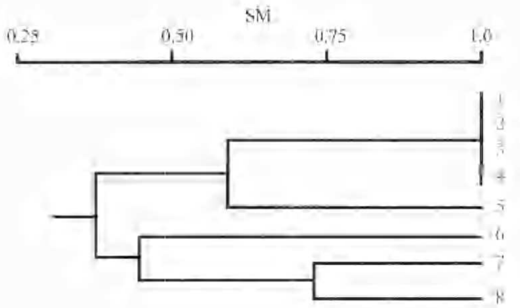

Fig. 1. UPGMA clustering of samples from a *Eucalyptus globulus* ssp. *bicostata* population at Mt Bryan in South Australia based on a simple matching coefficient (SM) measure of similarity calculated with RAPD markers. Samples 1-4 are from the 10m wide lignotuberous stand, while samples 5-8 are from individual trees in the vicinity of the stand.

a relatively large number of polymorphic bands, is very strong evidence for the clonality of samples 1-4. Assuming the growth rate of the *E. globulus* ssp. *bicostata* lignotuber was similar to that given by Tyson *et al.* (1998) for *E. risdonii* Hook. F. and *E. amygdalina* Labill. of about 2.5 mm/year, then it would have taken 4000 years for the *E. globulus* ssp. *bicostata* lignotuber to achieve its present size. This growth rate was comparable to that observed in *E. oleosa* F. Muell. ex Miq by Wellington *et al.* (1979), but greater than that obtained for a two metre diameter lignotuber of *E. coccifera* J. D. Hook (Head & Lacey 1988). We cannot say how old this individual really is, but it is probably much more than 200 years old. This population of *E. globulus* ssp. *bicostata* is therefore most likely to be natural and indeed an interesting remnant that deserves conservation. Although the site is being grazed by sheep (which would affect the rare understorey species and the eucalypt regeneration), the trees are long lived and not noticeably affected by grazing. Thus the population is not under any short term risk from the current land practices.

How did the *E. globulus* ssp. *bicostata* get established on Mt Bryan? One possibility is that it moved to this site through natural long distance seed dispersal. However, this eucalypt taxon, like most eucalypts, lacks adaptation for long distance seed dispersal (Potts & Wiltshire 1997). A related possibility is that this population was established from seed transported by aborigines. Another possibility is that it could have moved as ssp. *bicostata* pollen coming from afar and hybridising with an unknown resident eucalypt species, such as the related *E. goniocalyx* F. Muell. ex Miq. which occurs within 60 km of the site (see Potts & Reid 1988 for an example of this evolutionary mechanism). This would explain why the chloroplast DNA of this population is of a type very different from that encountered in other populations of *E. globulus* so far surveyed (Jackson *et al.* 1999). None of these hypotheses can be disproved. However, perhaps the simplest explanation for the occurrence of *E. globulus* ssp. *bicostata* at Mt Bryan is that the Victorian *E. globulus* ssp. *bicostata* populations were once connected to Mt Bryan at some time in the past. When this would have occurred is a matter for speculation. It is unlikely to have been in the last 35,000 years since the current aridity and the even greater aridity around the glacial maximum make it unlikely that the Murray Basin could have sustained *E. globulus* ssp. *bicostata* populations. It has often been assumed that this aridity may have been fairly constant from the Eocene to mid Miocene marine incursion into the Murray Basin (Marginson & Ladiges 1988). However, recent evidence from Lake Eyre suggests that there might have been wetter periods between 50,000 and 35,000 years BP (Magee & Miller 1998). Therefore, it is possible that during these or other previous wetter periods, an *E. globulus* ssp. *bicostata* forest could have been more or less continuous from Victoria to Mt Bryan in South Australia.

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References

CHATTAWAY, M. M. (1958) Bud development and lignotuber formation in eucalypts. *Aust. J. Bot.* **6**, 103-115.

DOYLE, J. J. & DOYLE, J. L. (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13-15.

GAIOTTO, F. A., BRAMUCCI, M. & GRATTAPAGLIA, D. (1997) Estimation of outcrossing rate in a breeding population of *Eucalyptus urophylla* with dominant RAPD and AFLP markers. *Theo. & Appl. Gen.* **95**, 842-849.

HEAD, M. J. & LACEY, C. J. (1988) Radiocarbon age determinations from lignotubers. *Aust. J. Bot.* **36**, 93-100.

JACKSON, H. D., STEANE, D. A., POTTS, B. M. & VAILLANCOURT, R. E. (1999) Chloroplast DNA evidence for reticulate evolution in *Eucalyptus* (Myrtaceae). *Mol. Ecol.* **8**, 739-751.

JACOBS, M. R. (1955) "Growth Habits of the Eucalypts" (Government printer, Canberra).

KENNINGTON, W. J., WAYCOTT, M. & JAMES, S. H. (1996) DNA fingerprinting supports notions of clonality in a rare mallee *Eucalyptus argutifolia*. *Mol. Ecol.* **5**, 693-696.

LACEY, C. J. (1983) Development of large plate like lignotubers in *Eucalyptus botryoides* Sm. in relation to environmental factors. *Aust. J. Bot.* **31**, 105-118.

_________ & JOHNSTON, R. D. (1990) Woody clumps and clumpwoods. *Ibid.* **38**, 299-334.

MAGEE, J. W. & MILLER, G. H. (1998) Lake Eyre palaeohydrology from 60ka to the present, beach ridges and glacial maximum aridity. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **144**, 307-329.

MARGINSON, J. C. & LADIGES, P. Y. (1988) Geographical variation in *Eucalyptus baxteri* s.l. and the recognition of a new species, *E. arenacea*. *Aust. Syst. Bot.* **1**, 151-170.

NESBITT, K. A., POTTS, B. M., VAILLANCOURT, R. E., WEST, A. K. & REID, J. B. (1995) Partitioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus* (Myrtaceae). *Hered.* **74**, 628-637.

_________, _________, _________ & REID, J. B. (1997) Fingerprinting and pedigree analysis in *Eucalyptus globulus* using RAPDs. *Silvae Genetica* **46**, 6-11.

POTTS, B. M. & REID, J. B. (1988) Hybridisation as a dispersal mechanism. *Evol.* **42**, 1245-1255.

_________ & WILTSHIRE R. J. E. (1997) Eucalypt genetics and genecology pp. 56-91 *In* Williams, J. & Woinarski, J. (Eds) "Eucalypt Ecology: Individuals to Ecosystems" (Cambridge University Press, Cambridge).

ROSSETTO, M., LUCAROTTI, F., HOPPER, S. D. & DIXON, K. W. (1997) DNA fingerprinting of *Eucalyptus graniticola* - A critically endangered relict species or a rare hybrid. *Hered.* **79**, 310-318.

_________, JEZIERSKI, G., HOPPER, S. D. & DIXON, K. W. (1999) Conservation genetics and clonality in two critically endangered eucalypts from the highly endemic south-western Australian flora. *Biol. Conserv.* **88**, 321-331.

ROHLF, F. J. (1993) 'NTSYS-pc, Numerical taxonomy and multivariate analysis system (v1.8)' (Department of ecology and evolution, State University of New York, New York).

SOKAL, R. R. & SNEATH, P. H. A. (1963) "Principles of numerical taxonomy" (Freeman, San Francisco).

SALE, M. M., POTTS, B. M., WEST, A. K. & REID, J. B. (1996) Molecular differentiation within and between *Eucalyptus risdonii*, *E. amygdalina* and their hybrids using RAPD markers. *Aust. J. Bot.* **44**, 559-569.

SKABO, S., VAILLANCOURT, R. E. & POTTS, B. M. (1998) Fine-scale genetic structure of *Eucalyptus globulus* ssp. *globulus* forest revealed by RAPDs. *Ibid* **46**, 583-594.

TYSON, M., VAILLANCOURT, R. E. & REID, J. B. (1998) Determination of clone size and age in a mallee eucalypt using RAPDs. *Ibid* **46**, 161-172.

VAILLANCOURT, R. E., SKABO, S. & GORE, P. (1998) Fingerprinting for quality control in breeding and deployment. *Aust. For.* **61**, 207-210.

WELLINGTON, A. B., POLACH, H. A. & NOBLE, I. R. (1979) Radiocarbon dating of lignotubers from mallee forms of *Eucalyptus*. *Search* **10**, 282-283.

WELSH, J. & MCCLELLAND, M. (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* **18**, 7213-7218.

WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. & TINGEY, S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Ibid* **18**, 6531-6535.